Delayed recognition of the European poplar shoot borer, *Gypsonoma aceriana* (Duponchel) (Lepidoptera: Tortricidae), in Canada

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ABSTRACT

The long-term presence of *Gypsonoma aceriana* (Duponchel) (Lepidoptera: Tortricidae: Olethreutinae), a European shoot-boring pest of poplars, was discovered in British Columbia during examination of cytochrome c oxidase I sequences of voucher specimens deposited in the Canadian Forest Service, Pacific Forestry Centre, arthropod reference collection. Originally identified as a species of *Epiblema*, *G. aceriana* was first recovered in BC in 1980, almost 20 years before it was reported in Washington State. DNA barcodes from both larval and adult collections are demonstrated to be conspecific with an adult collection from Great Britain. Preliminary surveys of early and late instar larval feeding damage in southwestern British Columbia demonstrate that this shoot borer is established on southern Vancouver Island and throughout the lower mainland of the province. The eastern-most collections made to date include locations near Yale in the Fraser Canyon, from the lower Coquihalla River watershed and from Hope.

Key Words: *Gypsonoma aceriana*, European poplar shoot borer, *Populus* spp., DNA barcoding, COI, nonindigenous species detection

INTRODUCTION

Two species of shoot-boring *Gypsonoma* (Tortricidae: Olethreutinae) that attack cottonwoods are known to occur in North America. The native cottonwood twig borer (CTB), *G. haimbachii* (Kearfott), ranges across eastern North America (Morris 1967; Solomon 1995), while the introduced European poplar shoot borer (EPSB), *G. aceriana* (Duponchel) (Fig. 1) has been reported only from western Washington State (Miller and LaGasa 2001; LaGasa *et al.* 2001). Both species exhibit similar life histories and damage in cottonwoods. Early instar larvae feed as leaf miners from late summer through fall. The second instar larvae of both species then spin hibernacula on the bark of stems or branches and overwinter. In the spring, larvae emerge from the hibernacula and bore into the developing green shoots, producing silk tunnels covered with frass and debris deposits at the entrances to their feeding galleries. At maturity, larvae of both species leave the damaged shoots and pupate in the leaf litter (Morris 1967; Solomon 1995; Miller and LaGasa 2001; LaGasa *et al.* 2001).

As frass and silk tubes similar to those caused by late instar EPSB and CTB had previously been noted on *Populus* species in British Columbia (BC), we undertook to determine: 1) which of the species of *Gyp-*

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Figure 1. Adult male (dorsal view) of *Gypsonoma aceriana* (Duponchel).

sonoma was present in BC; and, if either was present, 2) the host and geographic ranges of shoot-boring *Gypsonoma* spp. in the southwestern area of the province.

MATERIALS AND METHODS

Larval Rearings. During the spring of 2007, lateral and terminal new growth of black cottonwood, *Populus balsamifera* L. ssp. *trichocarpa* (Torr. & Gray ex Hook.) Brayshaw, and Lombardy poplar, *Populus nigra* L. *cv. 'italica'*, exhibiting feeding damage characteristic of EPSB were collected in the Greater Victoria area (Table 1) and reared until mature larvae emerged and dropped to the bottoms of the rearing tubes. Mature larvae were collected and placed into clean tubes with a 2-cm-deep layer of lightly moistened peat moss and held at room temperature until adult emergence.

Field Collections. In the fall of 2007, a roadside survey of *Populus* species was conducted in southwestern BC, along Highway (Hwy) 3 between Hope and Keremeos, along Hwy 3b from Keremeos to the junction of Hwy 97, along Hwy 97 north to Vernon, then west on Hwy 97 through Falkland to the junction of Hwy 1, and then west on Hwy 1 to Hope. Two branches were cut from all sampled roadside trees (to a maximum of five trees per location), and the undersides of all leaves were examined for leaf mines constructed by first or second instar larvae. A more detailed survey was conducted of the native and hybrid poplars managed by the British Columbia Ministry of Forests and Range at Kalamalka Research Station, Vernon, BC. All leaf mines were dissected, and any larvae recovered were preserved in 95% ethanol. Between 2007 and 2009, additional sites were surveyed for characteristic larval damage of *Gypsonoma* during other field activities. Locations of all sites positive or negative for damage or life stages of *Gypsonoma* are documented in Table 1 (positive collections) or Figure 2 (positive and negative sites).

Historical Collections. Voucher collections and historical records of the Forest Insect and Disease Survey held in the Canadian Forest Service (CFS) Reference collection (PFCA) at the Pacific Forestry Centre (PFC), Victoria, BC, were examined for previous collections of *Gypsonoma*.

Molecular and Morphological Identifications. DNA was extracted from legs removed from pinned adult specimens of *Gypsonoma* or from preserved first or second instar larvae extracted from leaf mines

Table 1.

Collection data for voucher specimens of *Gypsonoma aceriana* (Duponchel) deposited in the reference collection (PFCA) at Natural Resources Canada, Canadian Forest Service, Victoria, British Columbia.

Field Collection Number	Life Stage [No of specimens]	Collection Location	Latitude (dec. deg.)	Longitude (dec. deg.)	Collection Date [Date of adult emergence]	Host & Collection Data	Collector
FIDS 80-0271-01[1]	A	Saanich, Pacific Forestry Centre	48.460	-123.396	11-vi-1980	ex larva from new shoot *P. b. trichocarpa*	R. Duncan
FIDS 81-0384-01[1,2]	L [3]	Saanich, Royal Oak	48.475	-123.406	4-vii-1981	larva from new shoot *P. b. trichocarpa*	Duncan, Dobbin & Burnside
FIDS 81-0384-01[1]	A [3]	Saanich, Royal Oak	48.475	-123.406	4-vii-1981	ex larva from new shoot *P. b. trichocarpa*	Duncan, Dobbin & Burnside
DUN-06-0043-01[1]	A [8]	Saanich, Jolly Place	48.466	-123.383	26-v-2006	ex larva in new shoots, *P. b. trichocarpa*	R. Duncan
PFC-2006-0505	A [1]	Saanich, Pacific Forestry Centre	48.459	-123.397	17-vii-2006	MV light	L. Mavin & M. Young
PFC-2006-0907	A [1]	Saanich, Prospect Lake, Echo Dr.	48.461	-123.397	17-vii-2006	MV light	L. Mavin & M. Young
PFC-2006-1970	A [1]	Saanich, Pacific Forestry Centre	48.461	-123.397	24-viii-2006	MV light	L. Mavin & M. Young
PFC-2006-1971	A [1]	Saanich, Pacific Forestry Centre	48.515	-123.435	20-vii-2006	MV light	L. Mavin & M. Young
HUM-07-0136	L [1]	Saanich, Blenkinsop & Mackenzie	48.473	-123.351	14-vi-2007	larva from new shoot, *P. b. trichocarpa*	L.M. Humble
HUM-07-0136	A [1]	Saanich, Blenkinsop & Mackenzie	48.473	-123.351	[2-vii-2007]	ex larva from new shoot, *P. b. trichocarpa*	L.M. Humble
HUM-07-0144	A [3]	Saanich, Dupplin Rd	48.448	-123.378	[4-viii-2007]	ex larva in shoot *P. nigra cv. 'Italica'*	L.M. Humble
HUM-07-0361	L [20]	Saanich, Pacific Forestry Centre	48.459	-123.397	26-viii-2007	in mine along leaf midvein, *P. b. trichocarpa*	L.M. Humble

Table 1. (continued)

Field Collection Number	Life Stage [No of specimens]	Collection Location	Latitude (dec. deg.)	Longitude (dec. deg.)	Collection Date [Date of adult emergence]	Host & Collection Data	Collector
HUM-07-0362	L [10]	Saanich, Dupplin Rd	48.448	-123.378	26-viii-2007	in mine along leaf midvein, *P. nigra cv. 'italica'*	L.M. Humble
HUM-07-0374	L [65]	Saanich, U of Victoria	48.467	-123.318	5-ix-2007	in mines in leaf *P. b. trichocarpa*	L.M. Humble
HUM-07-0379	L [14]	Saanich, U of Victoria	48.467	-123.318	11-ix-2007	in mines in leaf *P. b. trichocarpa*	M. Bland
HUM-07-0382	L [50]	Saanich, U of Victoria	48.467	-123.318	18-ix-2007	mines on underside of leaf *P. b. trichocarpa*	M. Bland
HUM-07-0383	L [6]	Harrison Mills, N of Mill & Kilby Rds	49.236	-121.940	15-ix-2007	*Populus* hybrid: mines, underside of leaf	M. Bland
HUM-07-0384	L [2]	Hope, Memorial Park	49.381	-121.442	13-ix-2007	mines on underside of leaf, *P. nigra cv. 'italica'*	M. Bland
HUM-07-0385	L [1]	6 km S of Yale	49.516	-121.420	15-ix-2007	mines in leaf of *Populus* sp	M. Bland
HUM-08-0260	L [10]	Saanich, Pacific Forestry Centre	48.459	-123.397	12-v-2008	mining base of leaf petiole *P. b. trichocarpa*	L.M. Humble
HUM-09-0119	L [5]	Nanaimo Lakes Road	49.076	-123.884	29-v-2009	stem mine *P. b. trichocarpa*	L.M. Humble
HUM-09-0120	L [2]	Ladysmith	49.076	-123.967	29-v-2009	stem mine *P. b. trichocarpa*	L.M. Humble
HUM-09-0121	L [14]	Cowichan Bay	48.763	-123.644	29-v-2009	stem mine *P. alba*	L.M. Humble
HUM-09-0122	L [2]	Shawnigan Lake	48.614	-123.627	29-v-2009	stem mine *P. b. trichocarpa*	L.M. Humble
HUM-09-0123	L [8]	Cowichan Bay Road	48.743	-123.632	29-v-2009	stem mine *P. nigra cv. 'Italica'*	L.M. Humble
HUM-09-0124	L [4]	Othello Rd & Coquihalla Hwy	49.385	-121.319	1-vi-2009	stem mine *P. b. trichocarpa*	L.M. Humble
HUM-09-0365	L [2]	Carolin Mines Rd & Coquihalla Hwy	49.481	-121.252	18-vi-2009	mining new shoot *P. b. trichocarpa*	Humble & Noseworthy

[1] Previously identified as *Epiblema* n. sp.

[2] Freeze-dried larvae pinned with damaged shoots exhibiting frass-covered silken tubes

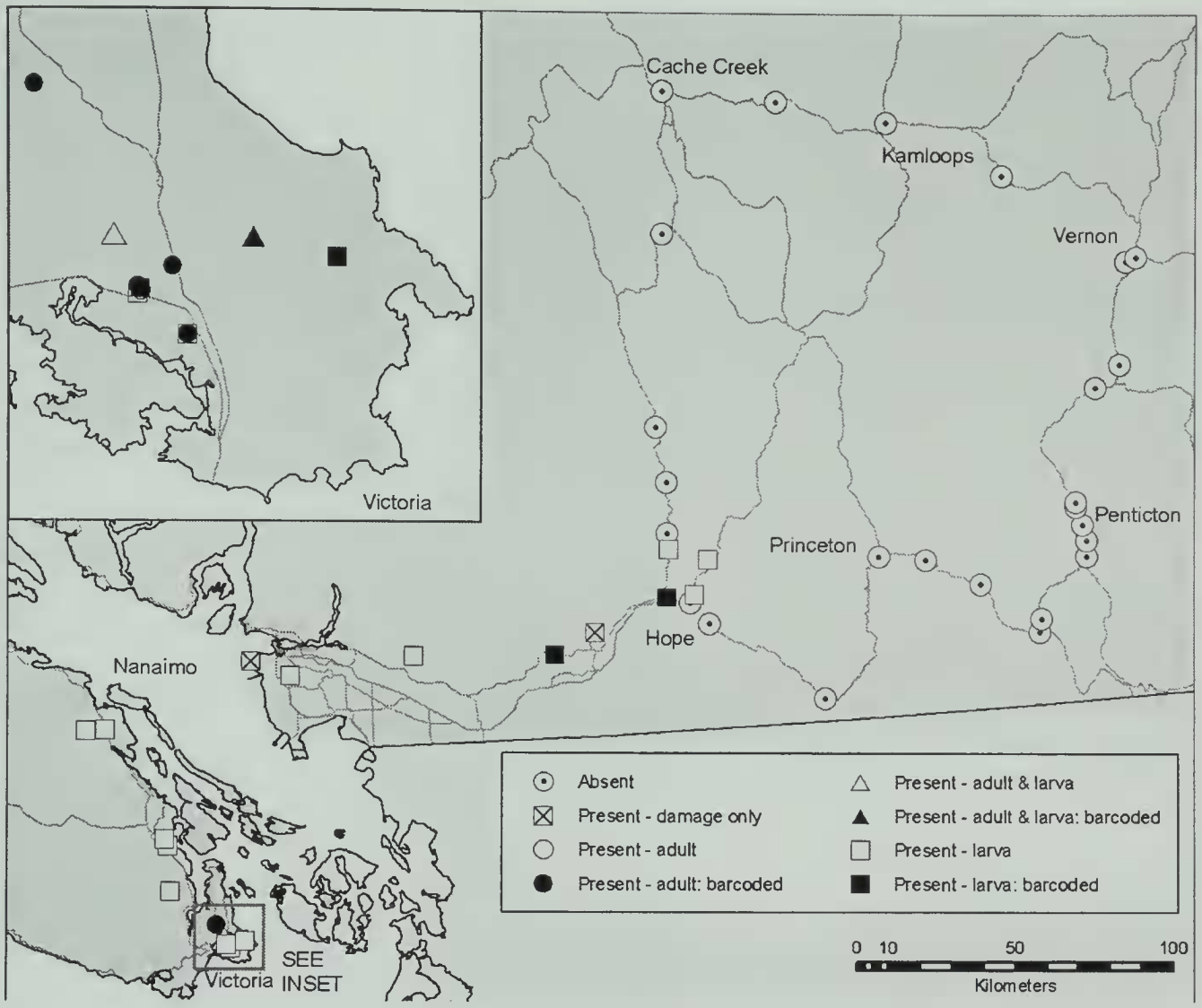

Figure 2. Locations surveyed and distribution of *Gypsonoma aceriana* (Duponchel) in southwestern British Columbia.

on *Populus* spp. (Table 1), and the barcode region of the mitochondrial gene cytochrome *c* oxidase I (COI) was amplified and sequenced using established protocols (Hajibabaei *et al.* 2005; Hebert *et al.* 2004; Ivanova *et al.* 2006; deWaard *et al.* 2008). BOLD-IDS, the identification engine of the Barcode of Life Database [see http://www.barcodinglife.org/views/idrequest.php], was used to assign tentative identifications for all sequences. Identifications were considered definitive if similarity scores of 100% were obtained. The barcode-assigned determinations of adults were confirmed morphologically through genitalic dissections. All specimens were deposited in the collection at the PFC, CFS, Victoria, BC (PFCA).

RESULTS AND DISCUSSION

The locations and hosts of all larval and adult collections of *Gypsonoma* examined in this study are documented in Table 1. We reared four adult *Gypsonoma* from two larval collections on *Populus* species in the Greater Victoria area. COI barcode sequences were obtained from four reared adults and 19 larvae recovered from various *Populus* species in collections made between 2006 and 2008. With the exception of one larva recovered from a leaf mine on black cottonwood in Victoria, all individuals collected were assigned to the species *Gypsonoma aceriana* (Duponchel) (Fig. 3) by the BOLD-IDS engine. A single larva of *Batrachedra praeangusta* (Haworth) recovered from an EPSB mine in the base of a leaf petiole in the spring of 2008 was also

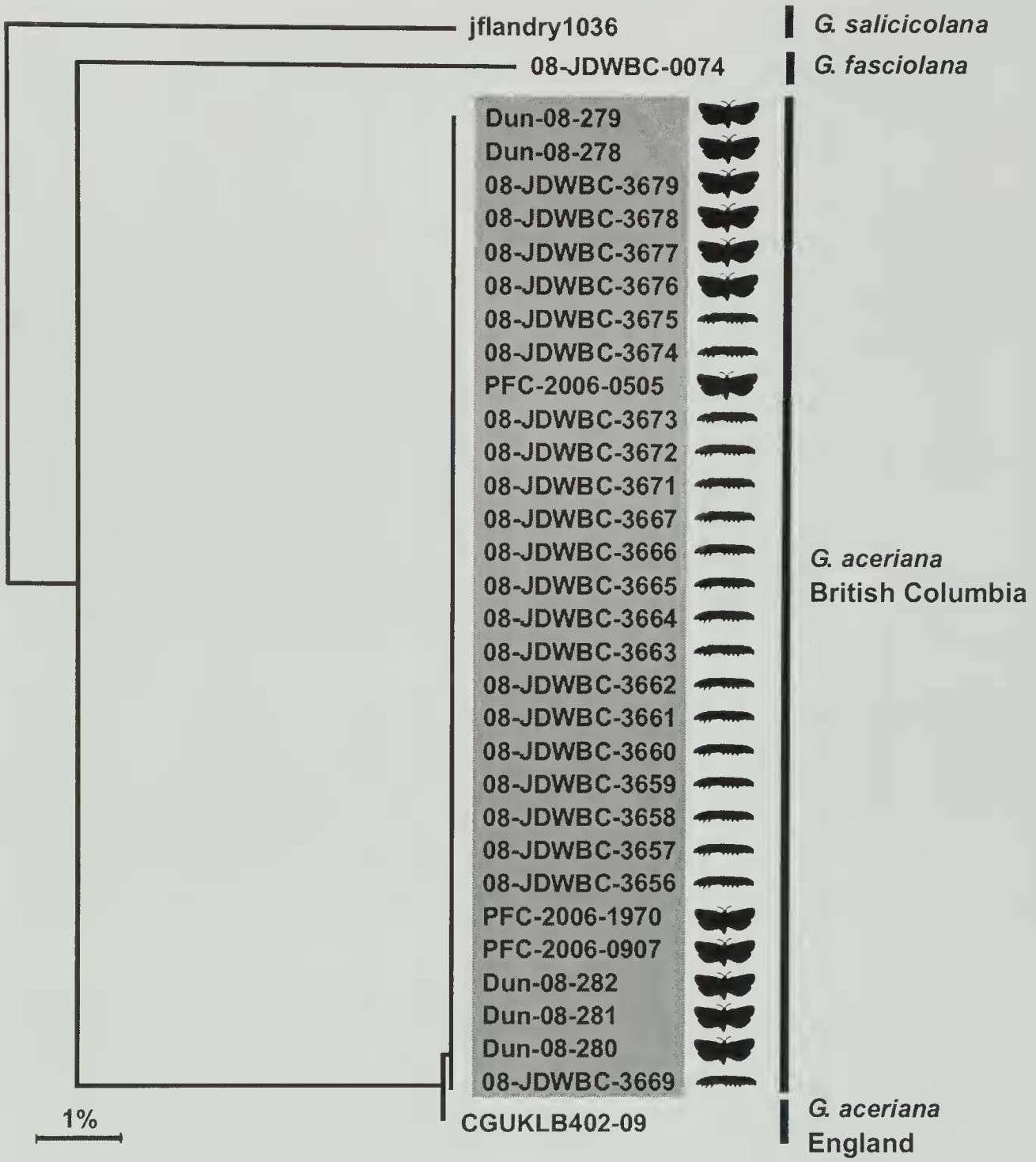

Figure 3. Taxon-ID tree of *Gypsonoma aceriana* (Duponchel) and congeners in British Columbia (modified from the tree output option of the Barcode of Life Database Identification System).

identified with BOLD-IDS. The neighbour-joining tree (based on Kimura-2-parameter distances (Kimura 1980) of COI sequences) that was generated by the BOLD-IDS engine also identified an additional nine COI sequences from voucher specimens deposited in PFCA (barcoded by LMH in order to build reference libraries of COI sequences) as those of *G. aceriana*. Five of the sequences were derived from moths originally identified as *Epiblema* sp. that had been reared from late instar larvae on *P. b. trichocarpa* in 2006, and an additional four sequences were obtained from moths captured in light traps on southern Vancouver Island in 2007 (Table 1). BOLD-IDS indicated a close match between the specimens barcoded from BC (Table 2) and a single

reference specimen from Great Britain (Fig. 3). Genitalic dissection of FIDS 80-0271-01 confirmed the identification of the adult moths as *G. aceriana*.

A re-examination of the Olethreutinae housed in PFCA led to the discovery of three additional pinned adults and three freeze-dried larvae pinned in association with damaged shoots bearing the frass-covered entrance tubes characteristic of EPSB. The adults had been submitted to the Canadian National Collection in Ottawa in 1981 (Lot. No. 81-42) for determination and were identified as "probably *Epiblema* sp.". That report noted that individuals of the other sex (males) were required for a definitive identification. A search of the historical Forest Insect and Disease Survey records (1949–1995) for all collections identified as *Epiblema* sp. on *Populus* species in BC produced five records, including the 1980 and 1981 collections (FIDS 1980-0271-01 and 1981-0384-01) that were confirmed to be *G. aceriana* in this study. No voucher specimens could be located for the remaining three collections; however, a series of damage photographs from one collection from the Cariboo (FIDS 1992-9-0083-01, Horse Lake, BC, 51.607°, -121.205°, 2-vi-1992, 6 larvae, D. White) document larvae identified as *Epiblema* boring in new shoots of *P.b. trichocarpa*. The absence of a dark prothoracic shield (present in *G. aceriana*) in the larval images from Horse Lake provides conclusive evidence that the damage was not caused by EPSB. Damage similar to that noted at Horse Lake was collected again in 1994 (FIDS 1994-9-0668-01, Blue Lead Cr, E end of Quesnel Lake, 52.625°, -120.375°, 23-vi-1994, *P. b. trichocarpa,* damage only, R. Erickson). As this collection is in close proximity to the previous collection, we suggest that it is unlikely to have been caused by EPSB. The final record (FIDS 1991-9-0718-01, Saanichton, CPFP Seed Orchard, 30-v-1991, 48.600°, -123.440°, damaged shoots of *Populus* sp.) also consisted of damage only, and thus cannot be conclusively attributed to EPSB.

The occurrence of *G. aceriana* in North America was first reported by Miller and LaGasa (2001) after a single male of EPSB was recovered near the port area of Seattle, WA, in 1998 and single males were detected at two additional locations in 1999. Subsequent surveys by LaGasa *et al.* (2001) demonstrated that EPSB was widely distributed in western Washington State. Voucher specimens (adults and freeze-dried larvae with associated damage) misidentified as *Epiblema* sp. (Tortricidae: Olethreutinae) deposited in PFCA demonstrate that EPSB was already present in southwestern BC by 1980, almost 20 years before the species was first recorded in Washington State.

As all but one late instar larvae recovered from mines in petioles and new shoots in the spring, as well as all early instar larvae recovered from mines on the underside of leaves in the fall, exhibited COI sequences identical to those of reared adults of *G. aceriana*, we feel confident that our visual survey records can be used to develop the first map of occurrences of EPSB in BC (Fig. 2). The full extent of ESPB distribution is yet unknown. It has been recovered on southeastern Vancouver Island from Victoria to Nanaimo, throughout the Fraser River valley, and as far inland as Yale in the Fraser Canyon and Carolin Mines Road along the Coquihalla Highway (Fig. 2). To date, evidence of damage caused by *Gypsonoma* sp. has not been found east of these locations. Although it is possible that low-level populations of EPSB may be present at some sites examined in the fall 2007 survey, we feel that the initial data are representative of EPSB distribution in those areas surveyed, as there was also no evidence of the more conspicuous debris-covered silken tubes constructed by the later instar larvae at the entrance to larval feeding tunnels that persist into the fall and winter (LaGasa *et al.* 2001). The results of this survey could be confirmed using pheromones identified by Booij and Voerman (1984).

Miller and LaGasa (2001) note that similarities between the poplar floras of Europe and North America, as well as the wide distribution of EPSB and its status as

Table 2.

Field collection numbers, Barcode of Life Database Sample ID and Process ID registration numbers, GenBank accession numbers, and life stage sampled for COI sequences of all individuals successfully barcoded. Unless otherwise noted, all barcoded specimens are *Gypsonoma aceriana*. Voucher specimens are deposited in the reference collection (PFCA) at Natural Resources Canada, Canadian Forest Service, Victoria, British Columbia.

Field Collection Number	BOLD Sample ID	BOLD Process ID	GenBank Accession No.	Life Stage
DUN-06-0043-01	Dun-08-278	DUNLP278-08	GQ845373	adult
DUN-06-0043-01	Dun-08-279	DUNLP279-08	GQ845374	adult
DUN-06-0043-01	Dun-08-280	DUNLP280-08	GQ845375	adult
DUN-06-0043-01	Dun-08-281	DUNLP281-08	GQ845376	adult
DUN-06-0043-01	Dun-08-282	DUNLP282-08	GQ845377	adult
HUM-07-0382	08-JDWBC-3656	LBCG3656-09	GQ845378	larva
HUM-07-0382	08-JDWBC-3657	LBCG3657-09	GQ845379	larva
HUM-07-0382	08-JDWBC-3658	LBCG3658-09	GQ845380	larva
HUM-07-0382	08-JDWBC-3659	LBCG3659-09	GQ845381	larva
HUM-07-0382	08-JDWBC-3660	LBCG3660-09	GQ845382	larva
HUM-07-0382	08-JDWBC-3661	LBCG3661-09	GQ845383	larva
HUM-07-0382	08-JDWBC-3662	LBCG3662-09	GQ845384	larva
HUM-07-0382	08-JDWBC-3663	LBCG3663-09	GQ845385	larva
HUM-07-0382	08-JDWBC-3664	LBCG3664-09	GQ845386	larva
HUM-07-0382	08-JDWBC-3665	LBCG3665-09	GQ845387	larva
HUM-07-0382	08-JDWBC-3666	LBCG3666-09	GQ845388	larva
HUM-07-0382	08-JDWBC-3667	LBCG3667-09	GQ845389	larva
HUM-07-0384	08-JDWBC-3672	LBCG3672-09	GQ845390	larva
HUM-07-0383	08-JDWBC-3673	LBCG3673-09	GQ845391	larva
HUM-07-0383	08-JDWBC-3674	LBCG3674-09	GQ845392	larva
HUM-07-0383	08-JDWBC-3675	LBCG3675-09	GQ845393	larva
HUM-08-0260	08-JDWBC-3669	LBCG3669-09	GQ845394	larva
HUM-08-0260	08-JDWBC-3671	LBCG3671-09	GQ845395	larva
HUM-07-0136	08-JDWBC-3676	LBCG3676-09	GQ845397	adult
HUM-07-0144-A	08-JDWBC-3677	LBCG3677-09	GQ845398	adult
HUM-07-0144-B	08-JDWBC-3678	LBCG3678-09	GQ845399	adult
HUM-07-0144-D	08-JDWBC-3679	LBCG3679-09	GQ845400	adult
PFC-2006-0505	PFC-2006-0505	LPVIA333-08	GQ845401	adult
PFC-2006-0907	PFC-2006-0907	LPV1A665-08	GQ845402	adult
PFC-2006-1970	PFC-2006-1970	LPV1B549-08	GQ845403	adult
PFC-2006-1971	PFC-2006-1971	LPVIB550-08	GQ845404	adult
HUM-08-0260	08-JDWBC-3670	LBCG3670-09	GQ845396	larva[1]

[1] Larva of *Batrachedra praeangusta* (Lepidoptera: Momphidae) recovered from frass and debris covered silk tunnel at the base of a leaf petiole

a pest in Europe, suggest that this shoot-borer could still become a significant pest of poplars in North America. Because of its lengthy presence in western North America, the pathway by which EPSB was introduced will never be determined. In BC, *G. aceriana* has been recorded from native black cottonwood, as well as two introduced poplars, European white poplar (*Populus alba* L.) and Lombardy poplar, that are widely planted as ornamentals or windbreaks in southwestern BC. The detection of EPSB in native poplars beyond urban forests (see Table 1, collection HUM-09-0365) suggests that natural spread has already occurred. The impact of EPSB on native poplars has not been evaluated in North America; however, in Belgium, Heymans *et al.* (1983) found that clones of *P. trichocarpa* and *P. trichocarpa* X *deltoides* hybrids were more susceptible to EPSB damage than were *P. deltoides* X *nigra* clones, with the earliest flushing clones sustaining the heaviest damage. The cryptic nature of the hibernacula of overwintering second instar larvae makes detection of this pest in horticultural or forest nursery stock very difficult. Prevention of continued spread of EPSB through movement of live plants or planting stock will require concerted efforts of the horticultural and forest silvicultural sectors.

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